Antibiotic Treatment of Persistent *Pseudomonas aeruginosa* Infections

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Introduction

Pseudomonas aeruginosa is a gram-negative opportunistic pathogen associated with infections due to severe burns, orthopedic implants, or in patients with cystic fibrosis. Treatment against these bacteria can be difficult because of the formation of biofilms and the increase in antibiotic resistance. *P. aeruginosa* lawn biofilms, when treated with tobramycin, showed generation of antibiotic tolerant and resistant cells. These cells are protected within the biofilm; as a result, they are often tolerant toward antibiotics. These tolerant cells can lead to the development of resistant cells. The tolerant cells under favorable growth conditions can re-establish the infection even in the presence of antibiotics.

Objective

The purpose of the study was to test different classes of antibiotics for the appearance and eradication of tolerant cells. To test for the efficacy of different classes of antibiotics, a bioluminescent strain of *Pseudomonas aeruginosa*, Xen41, was used. Xen41 was tested against various antibiotics to see if tolerant cells would appear. To test for the eradication of tolerant cells, tolerant colonies were formed using tobramycin and then the cells were tested against various antibiotic classes. The classes of antibiotics used in this study include fluoroquinolones, beta-lactams, polymyxins, and aminoglycosides. Preformed biofilms (24 hrs.) of Xen41 were formed on tryptic soy agar (TSA). Antibiotic filter discs were used to deliver the antibiotic. *In vivo* imaging system (IVIS) was used to test the response of Xen41 towards the antibiotics.

Definitions

Biofilm- a community of microorganisms that aggregate and adhere to surfaces. Microorganisms secrete extracellular polymeric substances allowing the biofilm to have increased tolerance towards antibiotics.

IVIS- *in vivo* imaging system; bioluminescent imaging system that allows imaging of bioluminescent bacteria. IVIS is also used to visualize the activity of the biofilm.

Tolerant Cells- population of bacteria that has decreased sensitivity toward antibiotics allowing it to grow in the presence of antibiotics. Tolerant colonies can return to being susceptible when re-exposed to the antibiotic.

Resistant Cells- population of bacteria that can grow in the presence of antibiotics due to resistant genes.

Replica Plating- the process of transferring bacteria from one plate to another. This maintains the patterns of colonies.

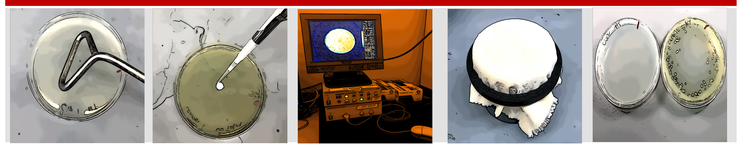
Methods

Materials	
P. aeruginosa Xen41	Filter discs
TSA plates	Replica plating stand
Antibiotic solutions	Velveteen cloth

Antibiotics

Antibiotic	Class	Mechanism of Action	Amount
Carbenicillin	Beta-lactam	Inhibits transpeptidase	100µg/disc
Meropenem	Beta-lactam	Inhibits transpeptidase	100µg/disc
Ciprofloxacin	Fluoroquinolone	Inhibits DNA gyrase	100µg/disc
Colistin	Polymyxin	Disrupts cellular membrane	100µg/disc
Gentamicin	Aminoglycoside	Binds 30S ribosome	100µg/disc
Tobramycin	Aminoglycoside	Prevent formation of 70S ribosome	100µg/disc

Formation of Tolerant Colonies

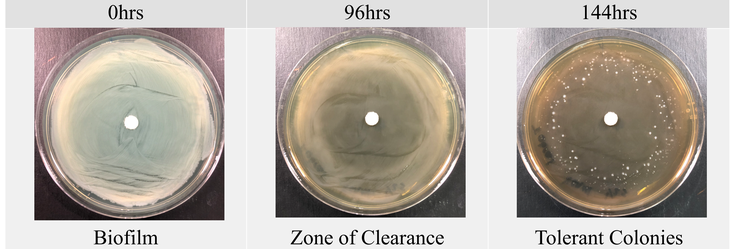


1. Spread lawn of Xen41 on TSA plate, incubated for 24hrs at 37°C
2. Place filter disc in the center and add antibiotic solution.
3. Take IVIS image every 24 hrs. for 7 days
4. Make antibiotic agar by spreading 100µg antibiotic on TSA plate
5. Replica plate using velveteen cloth onto TSA plate and TSA with respective antibiotics, incubate 24hrs; take IVIS image

Eradication of Tolerant Colonies

1. Spread lawn of Xen41 on TSA plate, incubated for 24hrs at 37°C
2. Place filter disc in the center and add tobramycin, incubated for 144hrs (7days); take IVIS image
3. Make antibiotic agar by spreading 100µg antibiotic on TSA plate
4. Replica plate using velveteen cloth onto TSA plate and TSA with antibiotic, incubate for 24hrs
5.Take IVIS image of the replica plates

Overview of the Experiments



Results

Figure 1- Formation of Tolerant Cells

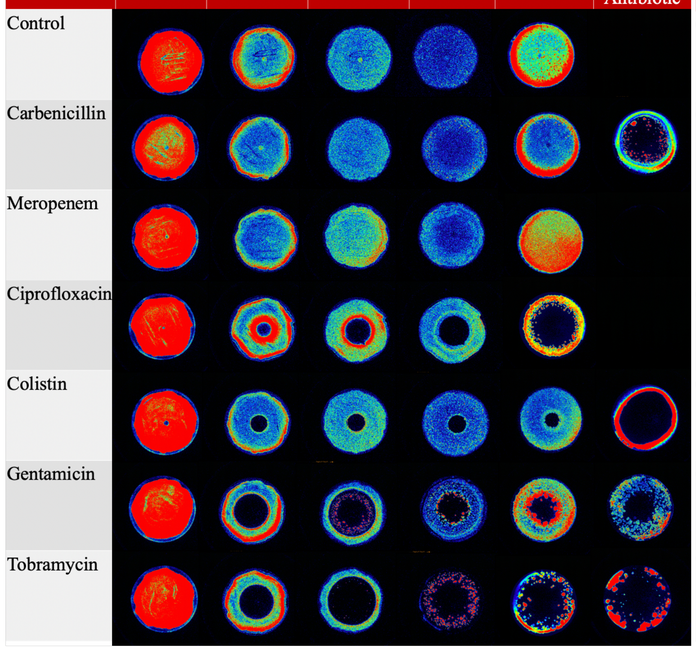


Figure 2- Eradication of Tolerant Colonies

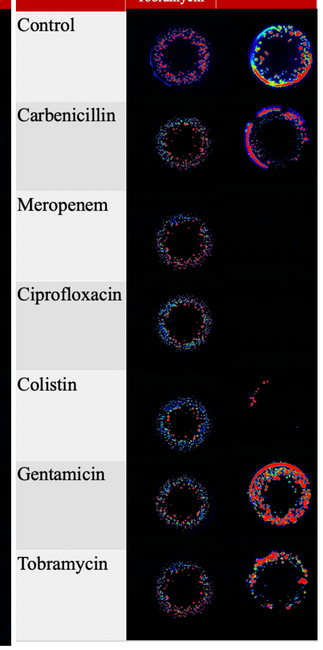


Figure 1- "Formation of Tolerant Cells" IVIS images showing the effect of antibiotic towards the biofilm

Figure 2- "Eradication of Tolerant Cells" IVIS image showing the activity of tolerant cells towards antibiotic

Conclusion and Analysis

The results showed that ciprofloxacin, colistin, gentamicin, and tobramycin had bactericidal effect, while carbenicillin and meropenem had a bacteriostatic effect on the biofilm. Of the antibiotics, gentamicin and tobramycin (aminoglycosides) showed the appearance of tolerant colonies. The tolerant cells were susceptible towards meropenem, ciprofloxacin, and colistin, while being resistant towards carbenicillin, gentamicin, and tobramycin. In conclusion, aminoglycosides allows the proliferation of tolerant cells and combination of antibiotics tobramycin with meropenem, ciprofloxacin, or colistin may be used to eradicate these tolerant cells.

Future Work

- Test other classes of antibiotics that are clinically used such as cephalosporins and monobactams in conjunction with combination of antibiotic
- Translate these finding on clinically isolates of *Pseudomonas aeruginosa*

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